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October 26, 2004

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APPLICATION NUMBER: ***PCT/US98/08857***
FILING DATE: ***May 01, 1998***

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COMMISSIONER OF PATENTS AND TRADEMARKS

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REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

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International Application No. PCT/US 98/08857

International Filing Date (01, 05. 98) 01 MAY 1998

PCT INTERNATIONAL APPLICATION

Name of receiving Office and "International Application"

Applicant's or agent's file reference (if desired) (12 characters maximum) CM1762F/JH

Box No. I TITLE OF INVENTION Fabric Care Compositions Comprising Cellulose Binding Domains

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

THE PROCTER & GAMBLE COMPANY
One Procter & Gamble Plaza
Cincinnati, Ohio 45202
US

☐ This person is also inventor.

Telephone No. 513-627-7025

Facsimile No. 513-627-6333

Teleprinter No.

State *(i.e. country)* of nationality: US

State *(i.e. country)* of residence: US

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

BAECK, Andre Cesar
Putsesteenweg 273
B-2820 Bonheiden
BE

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality: BE

State *(i.e. country)* of residence: BE

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

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Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

REED, T. David/HUGHETT, Eileen L.
The Procter & Gamble Company
5299 Spring Grove Avenue
Cincinnati, Ohio 45217
US

Telephone No. 513-627-7025

Facsimile No. 513-627-6333

Teleprinter No.

☐ Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Continuation of Box No. III FURTHER APPLICANTS AND/OR (FURTHER) INVENTORS

If none of the following sub-boxes is used, this sheet is not to be included in the request.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

SMETS, Johan
Bollenberg 79
B-3210 Lubbeek
BE

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality: BE

State *(i.e. country)* of residence: BE

This person is applicant for the purposes of:
- ☐ all designated States
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- ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

BOYER, Stanton Lane
12 Fall River Court
Fairfield, OH 45014
US

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality: US

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Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

This person is:
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- ☐ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

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Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

This person is:
- ☐ applicant only
- ☐ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality:

State *(i.e. country)* of residence:

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The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes; at least one must be marked)*:

Regional Patent

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The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)*

Box No. VI PRIORITY CLAIM Further priority claims are indicated in the Supplemental Box ☐

The priority of the following earlier application(s) is hereby claimed:

Country *(in which, or for which, the application was filed)*	Filing Date *(day/month/year)*	Application No.	Office of filing *(only for regional or international application)*
item (1)			
item (2)			
item (3)			

Mark the following check-box if the certified copy of the earlier application is to be issued by the Office which for the purposes of the present international application is the receiving Office (a fee may be required):

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Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(If two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):* ISA / EP

Earlier search *Fill in where a search (international, international-type or other) by the International Searching Authority has already been carried out or requested and the Authority is now requested to base the international search, to the extent possible, on the results of that earlier search. Identify such search or request either by reference to the relevant application (or the translation thereof) or by reference to the search request:*

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Box No. VIII CHECK LIST

This international application contains the following number of sheets:

1. request	: 04	sheets
2. description	: 61	sheets
3. claims	: 02	sheets
4. abstract	: 01	sheets
5. drawings	: 00	sheets
Total	: 68	sheets

This international application is accompanied by the item(s) marked below:

1. ☒ separate signed power of attorney (3)
2. ☒ copy of general power of attorney
3. ☐ statement explaining lack of signature
4. ☐ priority document(s) *identified in Box No. VI as item(s):*
5. ☒ fee calculation sheet
6. ☐ separate indications concerning deposited microorganisms
7. ☐ nucleotide and/or amino acid sequence listing (diskette)
8. ☒ other *(specify)*: postcard

Figure No. None of the drawings (if any) should accompany the abstract when it is published.

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

T. David Reed

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1. Date of actual receipt of the purported international application: 01 MAY 1998 (01.05.98)
2. Drawings: ☐ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:
4. Date of timely receipt of the required corrections under PCT Article 11(2):
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6. ☐ Transmittal of search copy delayed until search fee is paid

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Date of receipt of the record copy by the International Bureau:

GENERAL POWER OF ATTORNEY

We, The Procter & Gamble Company
One Procter & Gamble Plaza
Cincinnati, Ohio 45202
United States of America

hereby appoint:

Hasse, Donald E.	**29,387**
Reed, T. David	**32,931**
Hughett, Eileen L.	**34,352**
Guffey, Timothy B.	**41,048**
Hiland, Emelyn L.	**41,501**

all of 5299 Spring Grove Avenue, Cincinnati, Ohio 45217, as agents with full power of substitution to act on our behalf before all competent international authorities in connection with any and all international applications filed by us with either The United States Patent and Trademark Office or the PCT International Bureau of WIPO as receiving office for international applications filed under the Patent Cooperation Treaty, and to make or receive payments on our behalf.

Signed in Hamilton County, State of Ohio, U.S.A., the 16th day of March, 1998.

THE PROCTER & GAMBLE COMPANY

Jacobus C. Rasser
Assistant Secretary

STATE OF OHIO)
) **ss**
COUNTY OF HAMILTON)

On this 16 day of March, 1998, personally appeared before me Jacobus C. Rasser, to me personally known, who executed the foregoing instrument in my presence and acknowledged the execution thereof as his free and voluntary act and deed for the uses and purposes therein set forth and expressed.

NOTARIAL SEAL
STATE OF OHIO

Karen L. Pfeiffer
NOTARY PUBLIC
KAREN L. PFEIFFER
Notary Public, State of Ohio
My Commission Expires Sept. 15, 2002

PATENT COOPERATION TREATY
(Appointment of Agent or Common Respresentative)
POWER OF ATTORNEY

The undersigned applicant:

André Cesar Baeck
273 Putsesteenweg,
2820 Bonheiden, Belgium

(Complete name and address)

hereby appoint:

Hasse, Donald E.	**29,387**
Reed, T. David	**32,931**
Hughett, Eileen L.	**34,352**
Dostie, George E.	**39,173**

as agents to act on his/her behalf, with full power of substitution, before the competent international authorities filed by him/her with either the United States Receiving Office or The International Bureau of W.I.P.O. Receiving Office and to make or receive payments on our behalf.

Signed at STROMBEEK - BEVER
on this 03th day of OCTOBER 19 96

ANDRÉ CESAR BAECK

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PATENT COOPERATION TREATY
(Appointment of Agent or Common Representative)
POWER OF ATTORNEY

The undersigned applicant:

Johan (NMN) Smets
Bollenberg 79
3210 Lubbeek
Belgium

(Complete name and address)

hereby appoint:

	U.S. Registration No.
Hasse, Donald E.	**29,387**
Reed, T. David	**32,931**
Hughett, Eileen L.	**34,352**
Guffey, Timothy B.	**41,048**
Hiland, Emelyn L.	**41,501**

as agents to act on his/her behalf, with full power of substitution, before all competent international authorities in connection with any and all international application filed by him/her with either the United States Receiving Office or The International Bureau of W.I.P.O. Receiving Office and to make or receive payments on behalf of the undersigned.

Signed at Strombeek - Bever
on this 18 day of February 19 98

Johan Smets

Johan (NMN) Smets

PATENT COOPERATION TREATY
(Appointment of Agent or Common Representative)
POWER OF ATTORNEY

The undersigned applicant:

Stanton Lane Boyer
12 Fall River Court
Fairfield, OH 45014

(Complete name and address)

hereby appoints:

	U.S. Registration No.
Hasse, Donald E.	**29,387**
Reed, T. David	**32,931**
Hughett, Eileen L.	**34,352**

as attorney/agent to act on his behalf, with full power of substitution, before all competent international authorities in connection with any and all international applications filed by him with either the United States Receiving Office or The International Bureau of W.I.P.O. Receiving Office and to make or receive payments on behalf of the undersigned.

Signed at Cincinnati, Ohio
on this 28th day of January 1997.

Stanton Lane Boyer
Stanton Lane Boyer

ptopoa:boyer

FABRIC CARE COMPOSITIONS COMPRISING CELLULOSE BINDING DOMAINS

Field of the Invention

The present invention relates to fabric care compositions comprising an amino acid sequence comprising a Cellulose Binding Domain (CBD).

Background of the invention

Modern laundry detergent and/or fabric care compositions contain various detergent ingredients having one or more purposes in obtaining fabrics which are not only clean but also have retained appearance and integrity. Therefore, detergent components such as perfumes, soil release agents, fabric brightening agents, fabric softeners, chelants, bleaching agents and catalysts, dye fixatives and enzymes, have been incorporated in laundry detergent and/or fabric care compositions. One of such specific example is the use of enzymes, especially proteases, lipases, amylases and/or cellulases.

In particular, cellulase enzymes are used in detergent/fabric care compositions for their cleaning and fabric care benefits. The activity of cellulase is one in which cellulosic fibres or substrates are hydrolised by the cellulase and is depending on the particular function of the cellulase, which can be endo- or exo- cellulase, and on the respective hemicellulases. The cellulose structures are depolymerized or cleaved into smaller and thereby more soluble or dispersible fractions. This activity in particular on fabrics provides a cleaning, rejuvenating, softening and generally improved handfeel characteristics to the fabric structure.

It is known in the art through protein analysis that cellobiohydrolases, major endoglucanases and bacterial cellulases posses a bifunctional organisation in the form of a catalytical core domain and a smaller cellulose binding domain separated by a linker or flexible hinge stretch of amino acids.

In recent years, consumer desirability for fabric conditioning compositions has risen. Fabric softening compositions impart several desirable properties to treated garments including softness and static control. Fabric softness of laundered garments is typically achieved by delivering a quaternary ammonium compound to the surface of the fabric. Consumer desirability for durable press fabric garments, particularly cotton fabric garments, has also risen. Durable press garments include those garments which resist wrinkling of the fabric both during wear and during the laundering process. Durable press garments can greatly decrease the hand work associated with laundering by eliminating ironing sometimes necessary to prevent wrinkling of the garment. However, in most commercially available durable press fabrics, the fabric's ability to resist wrinkling is reduced over time as the garment is repeatedly worn and laundered. Furthermore, coloured garments have a tendency to wear and show appearance losses. A portion of this colour loss may be attributed to abrasion in the laundering process, particularly in automatic washing machines and automatic laundry dryers. Moreover, tensile strength loss of fabric appears as an unavoidable result of mechanical / chemical action due to use / wearing or washing.

As indicated above, there is a continuous need for a fabric care composition, which can provide fabric softness and provide, refurbish or restore tensile strength, anti-wrinkle, anti-bobbling and anti-shrinkage properties to fabrics, as well as provide static control, colour appearance and fabric anti-wear properties and benefits.

The above objective has been met by formulating fabric care compositions comprising one or more amino acid sequence(s) comprising a Cellulose Binding Domain (CBD).

Enzymes linked to Cellulose Binding Domains are described in the art : WO 91/10732 novel derivatives of cellulase enzymes combining a core region derived from an endoglucanase producible by a strain of Bacillus spp., NICMB 40250

with a CBD derived from another cellulase enzyme or a combining a core region derived from another cellulase enzyme with a CBD derived from said endoglucanase, for improved binding properties. WO94/07998 describes cellulase variants of a cellulase classified in family 45, comprising a CBD, a Catalytically Active Domain (CAD) and a region linking the CBD to the CAD, wherein one or more amino acid residues have been added, deleted or substituted and /or another CBD is added at the opposite end of the CAD. WO95/16782 relates to the cloning and high level expression of novel truncated cellulase proteins or derivatives thereof in *Trichoderma longibrachiatum* comprising different core regions with several CBDs. WO97/01629 describes cellulolytic enzyme preparation wherein the mobility of the cellulase component may be reduced by adsorption to an insoluble or soluble carrier e.g. via the existing or newly introduced CBD. WO97/28243 describes a process for removal or bleaching or soiling or stains from cellulosic fabrics wherein the fabric is contacted in aqueous medium with a modified enzyme which comprises a catalytically active amino acid sequence of a non-cellulolytic enzyme selected from amylases, proteases, lipases, pectinases and oxidoreductases, linked to an amino acid sequence comprising a cellulose binding domain and a detergent composition comprising such modified enzyme and a surfactant.

However, none of these documents disclose fabric care compositions comprising one or more amino acid sequence(s) comprising a Cellulose Binding Domain, for fabric care benefits.

Summary of the invention

The present invention relates to fabric care compositions comprising one or more amino acid sequence(s) comprising a cellulose binding domain, providing fabric care.

In a further embodiment, the present invention relates to fabric care compositions wherein the amino acid sequence comprising a cellulose binding domain, is linked to a softening protein.

The fabric care compositions of the present invention can further comprise a softening ingredient selected from cationic surfactants, a transferase enzyme and/or clays.

Detailed description of the invention

An essential element of the fabric care compositions of the present invention is an amino acid sequence comprising a cellulose binding domain. This amino acid sequence comprising a cellulose binding domain (CBD) can be a naturally occuring sequence or can be a modified sequence. This modified sequence corresponds to cross-linked CBDs, i.e. amino acid sequences comprising a cellulose binding domain which are cross-linked to each other or corresponds to CBDs further linked to a softening protein, i.e. an amino acid sequence comprising a cellulose binding domain linked to a softening protein.

Indeed, the present invention encompasses several embodiments :

In a first embodiment, the present invention relates to a fabric care composition comprising one or more amino acid sequence(s) comprising a cellulose binding domain. It has been surprisingly found that such compositions comprising one or more CBDs, provide softening, abrasion resistance and pilling prevention. Indeed, without wishing to be bound by theory, it is believed that the CBDs adsorb on the fibres of the fabric. CBDs are proteins and therefore provide softening. Moreover, such adsorbed CBDs protect the fabric's fibers and thereby prevent the fibrillation of the cellulosic fibres.

In a second embodiment, the present invention encompasses a fabric care composition comprising cross-linked amino acid sequences comprising a cellulose binding domain. It has been surprisingly found that such cross-linked CBDs provide softness, prevent the fibrillation of cellulosic fibers as indicated above and also restore tensile strength, prevent the apparition of wrinkles, and increase the hydrophilicity of synthetic fibers. Indeed, without wishing to be bound by theory, it is believed that each cross-linked CBD adsorbs at opposite sites of the damaged fibers and thereby restore tensile strenght.

In a third embodiment, the present invention relates to a fabric care composition comprising the above described CBDs which are further linked to a softening protein. Without wishing to be bound by theory, it is believed that the addition of a cellulose binding domain to a softening protein, allows a higher concentration of the softening protein onto the fabric, i.e. a closer and/or more lasting contact, resulting in a more efficient activity. Such modified softening proteins have an increased affinity (relative to unmodified softening protein) for binding to a cellulosic fabric or textile.

The above described fabric care composition may further comprise a softening ingredient selected from cationic surfactants, a transferase enzyme and/or clays.

Cellulose Binding Domain (CBD)

In the present context, the terms "amino acid sequence comprising a CBD or Cellulose Binding Domain or CBD" are intended to indicate an amino acid sequence capable of effecting binding of the cellulase to a cellulosic substrate (e.g. as described in P. Kraulis et al., Determination of the three-dimensional structure of the C terminal domain of cellobiohydrolase I from Trichoderma reesei. A study using nuclear magnetic resonance and hybrid distance geometry-dynamically simulated annealing. Biochemistry 28:7241-7257, 1989). The classification and properties of cellulose binding domains are presented in P. Tomme et al., in the symposium "Enzymatic degradation of insoluble polysaccharides" (ACS Symposium Series 618, edited by J.N. Saddler and M.H. Penner, ACS, 1995).

Cellulose-binding (and other carbohydrate-binding) domains are polypeptide amino acid sequences which occur as integral parts of large polypeptides or proteins consisting of two or more polypeptide amino acid sequence regions, especially in hydrolytic enzymes (hydrolases) which typically comprise a catalytic domain containing the active site for substrate hydrolysis and a carbohydrate-binding domain for binding to the carbohydrate substrate in question. Such enzymes can comprise more than one catalytic domain and one, two or three carbohydrate-binding domains, and they may further comprise one or more

polypeptide amino acid sequence regions linking the carbohydrate-binding domain(s) with the catalytic domain(s), a region of the latter type usually being denoted a "linker".

Examples of hydrolytic enzymes comprising a cellulose-binding domain are cellulase, xylanases, mannanases, arabinofuranosidases, acetylesterases and chitinases. "Cellulose-binding domains" have also been found in algae, e.g. in the red alga porphyra purpurea in the form of a non-hydrolytic polysaccharide-binding protein [see P. Tomme et al., Cellulose-binding domains - Classification and Properties in Enzymatic Degradation of Insoluble Carbohydrates , John N. Saddler and Michael H. Penner (Eds.), ACS Symposium Series, No. 618 (1996)]. However, most of the known CBDs (which are classified and referred to by P. Tomme et al. (op. cit.) as "cellulose-binding domains"] derive from cellulases and xylanases.

In the present context, the term "cellulose-binding domain" is intended to be understood in the same manner as in the latter reference (P. Tomme et al., op. cit.) The P. Tomme et al. reference classifies more than 120 "cellulose-binding domains" into 10 families (I-X) which may have different functions or roles in connection with the mechanism of substrate binding. However, it is to be anticipated that new family representatives and additional families will appear in the future.

In proteins/polypeptides in which CBDs occur (e.g. enzymes, typically hydrolytic enzymes such as cellulases), a CBD may be located at the N or C terminus or at an internal position.

The part of a polypeptide or protein (e.g. hydrolytic enzyme) which constitutes a CBD per se typically consists of more than about 30 and less than about 250 amino acid residues. For example, those CBDs listed and classified in Family I in accordance with P. Tomme et al. (op. cit.) consist of 33-37 amino acid residues, those listed and classified in Family IIa consist of 95-108 amino acid residues, those listed and classified in Family VI consist of 85-92 amino acid residues, whilst one CBD (derived from a cellulase from Clostridium thermocellum) listed and classified in Family VII consists of 240 amino acid residues. Accordingly, the molecular weight of an amino acid sequence

constituting a CBD per se will typically be in the range of from about 4kD to about 40kD, and usually below about 35kD.

Cellulose binding domains can be produced by recombinant techniques as described in H. Stålbrand et al., Applied and Environmental Microbiology, Mar. 1995, pp. 1090-1097; E. Brun et al., (1995) Eur. J. Biochem. 231, pp. 142-148; J.B. Coutinho et al., (1992) Molecular Microbiology 6(9), pp. 1243-1252

In order to isolate a cellulose binding domain of, e.g. a cellulase, several genetic engineering approaches may be used. One method uses restriction enzyme to remove a portion of the gene and then to fuse the remaining gene-vector fragment in frame to obtain a mutated gene that encodes a protein truncated for a particular gene fragment. Another method involves the use of exonucleases such as Ba131 to systematically delete nucleotides either externally from the 5' and the 3' ends of the DNA or internally from a restricted gap within the gene. These gene-deletion methods result in a mutated gene encoding a shortened gene molecule whose expression product may then be evaluated for substrate-binding (e.g. cellulose-binding) ability. Appropriate substrates for evaluating the binding ability include cellulosic materials such as Avicel ™ and cotton fibres. Other methods include the use of a selective or specific protease capable of cleaving a CBD, e.g. a terminal CBD, from the remainder of the polypeptide chain of the protein in question.

Once a nucleotide sequence encoding the substrate-binding (carbohydrate-binding) region has been identified, either as cDNA or chromosomal DNA, it may then be manipulated in a variety of ways to fuse it to a DNA sequence encoding the amino acid sequence of interest. The DNA fragment encoding the carbohydrate-binding amino acid sequence, and the DNA encoding the amino acid sequence of interest are then ligated with or without a linker. The resulting ligated DNA may then be manipulated in a variety of ways to achieve expression. Preferred microbial expression hosts include certain Aspergillus species (e.g. A. niger or A. oryzae), Bacillus species, and organisms such as Escherichia coli or Saccharomyces cerevisiae.

Preferred CBDs for the purpose of the present invention are selected from the group consisting of : CBDs CBHII from *Trichoderma reesei*, CBDs CenC,

CenA and Cex from *Cellulomonas fimi*, CBD CBHI from *Trichoderma reesei*, CBD Cellulozome from *Clostridium cellulovorans*, CBD E3 from *Thermonospora fusca*, CBD-dimer from *Clostridium stecorarium* (NCIMB11754) XynA, CBD from *Bacillus agaradherens* (NCIMB40482) and/or CBD family 45 from *Humicola insolens*. More preferred CBD for the purpose of the present invention are the CBD CenC from *Cellulomonas fimi*, CBD Cellulozome from *Clostridium cellulovorans* and/or the CBD originating from the fungal *Humicola Insolens* cellulase sold under the tradename "Carezyme" by Novo Nordisk A/S. Carezyme is an endoglucanase from family 45, derived from *Humicola insolens* DSM1800, having a molecular weight of about 43kDa and exhibiting cellulolytic activity

The fabric care composition can comprise one or more of the above described CBDs, any of these CBDs which are cross linked and/or further linked to a softening protein and/or mixtures thereof. The cross-linked CBDs of the present invention can comprise the amino acid sequence comprising from 2 to 50, preferably 2 to 10 cellulose binding domains. The CBDs comprised in the fabric care compositions of the present invention may originate from different sources. The CBDs, the cross-linked CBDs and/or the CBD linked to a softening protein are generally comprised in the fabric care compositions of the present invention at a level of from 0.01% to 10% and preferably from 0.1% to 6% and in a concentrated fabric care composition, from 0.2% to 30%, from 2% to 20% by weight.

For example, as described by M. Linder et al in The Journal of Biological Chemistry, Vol. 271, No. 35, Issue of August, pp 21268-21272, 1996, a double CBD by fusing the N-terminal CBD of T. reesei CBHII to the C-terminal CBD of CBHI by a linker region of 24 amino acids can be constructed. The linker region contains three amino acid residues from the natural CBHII linker followed by 21 amino acid residues from the natural CBHI linker. The double CBD was cloned an produced in Escherichia coli. It has been observed that the two domains interact during binding on cellulose, resulting in a higher binding affinity of the double CBD than either of the two single domains by themselves.

Construction of the Double CBD Peptide - All DNA manipulations were performed using standard protocols. The gene constructions were first assembled into the vector pSP73 (Promega). The coding region for the pelB signal sequence of Erwinia carotovora was fused in frame with the coding region

of the first 41 N-terminal residues of CBHII derived from the plasmid pTTc9, which in turn was linked to the coding region of the last 57 residues of CBHI derived from the plasmid pTTcI). For expression in E. coli, the construction was inserted into the expression vector pKK223-3, containing the isopropyl-β-D-thiogalactopyranoside-inducible tac promoter. The nucleotide sequence of the final construct was verified by sequencing.

Fermentation - The E. coli cultivations for producing the double CBD were carried out in a Chemap CMF laboratory fermenter with a working volume of 1.5 liters. A pH of 7 was maintained throughout the fermentation, and the rate of agitation was controlled to maintain constant dissolved oxygen levels. During the exponential growth phase (15-20 h after inoculation), isopropyl-β-D-thiogalactopyranoside was added to a final concentration of 0.5 mM to induce gene expression. The fermentation was continued until maximal levels of product had been reached (20-30 h).

Purification of the Double CBD - Culture supernatant was centrifuged (10.000 rpm, 45 min) and mixed with an equal volume of 20 mM phosphate buffer, pH 8.0, containing 1.5 M ammonium sulfate and kept at 4°C overnight. Precipitation was removed by centrifugation as above, and the supernatant was filtered through a 0.45μm Durapore (Millipore Corp.) membrane. The clarified supernatant was then loaded onto a butyl-Sepharose 4B column (Pharmacia Biotech Inc.) previously equilibrated with 10 mM phosphate buffer, pH 8.0, containing 0.75 M ammonium sulfate. The column was then washed with equilibration buffer, and the bound protein was eluted with 10 mM phosphate buffer, pH 8.0. The eluted peak fractions were loaded onto a Source RPC column (Pharmacia) equilibrated with Milli-Q water:trifluoroacetic acid (1000:1). Bound peptide was eluted with an increasing linear gradient of acetonitrile:trifluoroacetic acid (1000:1). The purified peptide was then lyophilized. During all purification steps the double CBD was identified by the monoclonal antibody CI-89, which is specific toward the CBHI CBD. All chromatographic steps were run on a fast protein liquid chromatography (Pharmacia) system.

Proteolytic Cleavage - The lyophilized peptide was redissolved in 100 mM Tris buffer, pH 8.2 (2 mg/ml) and 10 units of immobilized trypsin (Sigma T-4019) added per mg of peptide. The suspension was incubated at 37°C overnight, purified by the chromatography on Source RPC media (see above), and then

lyophilized. The cleavage products were characterized and identified by amino acid analysis and MALDI-MS.

Analytical Techniques for the CBD Peptides - Purity control and quantification of CBD peptides was performed by RP-HPLC. A Pro-PepVydac C18 analytical column was used with gradient elution with water:trifluoroacetic acid (1000:1) to acetonitrile:trifluoroacetic acid (1000:1). Absorbance at 225 nm was used for detection. Quantification of peptide in the culture supernatant was also possible by this technique.

Production and Purification of the Double CBD - The amino acid sequence of the processed form of the double CBD is shown in Fig.1 (M. Linder et al in The Journal of Biological Chemistry, Vol. 271, No. 35, Issue of August, pp 21268-21272, 1996). In fermentor cultivations of the E. coli WCM105 strain 60-80 mg/liter of the peptide was secreted into the culture medium, yielding 40-50 mg/liter of pure peptide (see Fig. 2 - M. Linder et al in The Journal of Biological Chemistry, Vol. 271, No. 35, Issue of August, pp 21268-21272, 1996). The identity and correct processing of the peptide was verified by amino acid composition analysis. The peptide identity was also confirmed by Western blotting with a monoclonal antibody specific for the CBHI CBD.

The fabric care composition can also comprise the above described amino acid sequences further linked to a softening protein.

It has been found that enzyme proteins surprisingly show high adsorbability to a cellulosic fiber (EP 687 729). Enzyme proteins used in the present invention are those generically defined as a class of proteins having a particular structure for catalytic action. In other words, all proteins that possess a structure for catalytic action can be used, whether or not they exhibit catalytic action. However, enzyme proteins suitable for the purpose of the present invention are inactive. Inactivation can occur for example by inhibition, by the distortion of the three-dimensional structure for example by thermal or chemical means. When proteins other than enzyme proteins are used, the effects of the present invention may be obtained to some extent, but sufficient effect may not be achieved. Enzyme proteins have different biological origins: animal, plant and microbial origins. Enzyme proteins of any origin are usable for the present invention.

Such enzyme proteins, as classified on the basis of enzyme reaction type, include hydrolases, lyases, oxidoreductases, ligases, transferases and isomerases, all of which are usable for the present invention. A preference is given to hydrolases, exemplified by proteases (peptidase), glucosidases such as cellulase and amylase, and esterases such as lipase.

The molecular weight of the enzyme protein is preferably not lower than 10,000, more preferably in the range of from 20,000 to 300,000. Being not lower than 10,000 in molecular weight, some enzyme proteins cannot penetrate the single fiber/monofilament (lamella structure) of cellulosic fibers such as a natural cellulose fiber and rayon. Also, they may not penetrate the monofilament of synthetic fibers, because the monofilament internal structure is dense. Enzyme adsorption sites of cellulosic fibers and synthetic fibers are therefore limited to the surface of the single fiber/monofilament.

Preferably the softening enzyme protein will be chosen from inactive enzyme comprising a CBD in nature, such as cellulase, xylanases, mannanases, arabinofuranosidases, acetylesterases and chitinases.

JP01280079 describes another type of softening proteins that can be used in the fabric care compositions of the present invention. These softening proteins are polyamino acid resin solution that adhere to synthetic, semi synthetic or cotton fabrics and thereby provide softness. These polyamino acids are preferably alpha amino acid such as glutamic acid, glycine, ornithine, mono- or co-polymer such as poly-gamma-L-glutamate.

Also suitable for the present invention are the C18 alkyl quaternary wheat protein derivatives sold under the tradename Coltide HQS by Croda Colloids Ltd. These wheat proteins derivatives generally included at levels of 0.04% to 0.2% by weight, are known to provide great conditioning effect, i.e. to provide great handfeeling, softeness, to prevent fibres erosion of cotton and wool fabrics and to increase the lubricity of wool fibres.

Such amino acid sequence comprising one ore more CBDs and/or being further linked to a softening proteins (referred to as CBD hybrids and/or softening protein hybrids) can be prepared and purified by methods known in the art [see, e.g., WO 90/00609, WO 94/24158 and WO 95/16782, as well as Greenwood et al., Biotechnology and Bioengineering 44 (1994) pp. 1295 - 1305]. The

production of enzymes hybrid is given in WO 91/10732 wherein novel derivatives of cellulase enzymes combining a core region derived from a Bacillus NICB 40250 endoglucanase with a CBD derived from another cellulase enzyme or a combining a core region derived from another cellulase enzyme with a CBD derived from a Bacillus NICB 40250 endoglucanase, are constructed. WO 95/16782 describes the combinations of different core regions with several CBD and the cloning and high level expression of these novel truncated cellulase proteins or derivatives thereof, in Trichoderma longibrachiatum.

The CBD hybrid and/or softening protein hybrid may, e.g., be prepared by transforming into a host cell a DNA construct comprising at least a fragment of DNA encoding the cellulose-binding domain ligated, with or without a linker, to a DNA sequence encoding the other cellulose binding domain and/or softening protein of interest, and growing the transformed host cell to express the fused gene. One relevant, but non-limiting, type of recombinant product (CBD hybrid and/or softening protein hybrid) obtainable in this matter - often referred to in the art as a "fusion protein" - may be described by one of the following general formulae:

A-CBD-MR-X-B

A-X-MR-CBD-B

In the latter formulae, CBD is an amino acid sequence comprising at least the cellulose-binding domain (CBD) per se.

MR (the middle region; a linking region) may be a bond, or a linking group comprising from 1 to about 100 amino acid residues, in particular of from 2 to 40 amino acid residues, e.g. from 2 to 15 amino acid residues. MR may, in principle, alternatively be a non-amino-acid linker (See below).

X is an amino acid sequence comprising another cellulose binding domain and/or an amino acid sequence comprising the above-mentioned, inactive sequence of amino acid residues of a polypeptide encoded by a DNA sequence encoding the softening protein of interest.

The moieties A and B are independently optional. When present, a moiety A or B constitutes a terminal extension of a CBD or X moiety, and normally comprises one or more amino acid residues.

It will thus, inter alia, be apparent from the above that a CBD in a softening protein hybrid of the type in question may be positioned C-terminally, N-

terminally or internally in the softening protein hybrid. Correspondingly, an X moiety in a softening protein hybrid of the type in question may be positioned N-terminally, C-terminally, or internationally in the softening protein hybrid.

Softening protein hybrids of interest in the context of the invention include softening protein hybrids which comprise more than one CBD, e.g. such that two or more CBDs are linked directly to each other, or are separated from one another by means of spacer or linker sequences (consisting typically of a sequence of amino acid residues of appropriate length). Two CBDs in an softening protein hybrid of the type in question may, for example, also be separated from one another by means of an -MR-X- moiety as defined above.

One or more cellulose binding domain can be linked to the N-terminal and/or C-terminal parts of the cellulase core region. Any part of a CBD can be selected, modified, truncated etc.

Preferably, attention will be paid in the construction of CBD hybrid and/or softening protein hybrids of the type in question to the stability towards proteolytic degradation. Two- and multi-domain proteins are particularly susceptible towards proteolytic cleavage of linker regions connecting the domains. Proteases causing such cleavage may, for example, be subtilisins, which are known to often exhibit broad substrate specificities [see, e.g. : Grøn et al., Biochemistry 31 (1992), pp. 6011-6018; Teplyakov et al., Protein Engineering 5 (1992), pp. 413-420]. Glycosylation of linker residues in eukaryotes is one Nature's ways of preventing proteolytic degradation. Another is to employ amino acids which are less favoured by the surrounding proteases. The length of the linker also plays a role in relation to accessibility by proteases. Which "solution" is optimal depends on the environment in which the softening protein hybrid is to function. When constructing new CBD hybrid and/or softening protein hybrid molecules, attention will be preferably paid to the linker stability.

Plasmids

Preparation of plasmids capable of expressing fusion proteins having the amino acid sequences derived from fragments of more than one polypeptide is well-known in the art (see, for example, WO 90/00609 and WO 95/16782). The expression cassette may be included within a replication system for episomal

maintenance in an appropriate cellular host or may be provided without a replication system, where it may become integrated into the host genome. The DNA may be introduced into the host in accordance with known techniques such as transformation, microinjection or the like.

Once the fused gene has been introduced into the appropriate host, the host may be grown to express the fused gene. Normally it is desirable additionally to add a signal sequence which provides for secretion of the fused gene. Typical examples of useful genes are:

1) Signal sequence -- (pro-peptide) -- carbohydrate-binding domain -- linker -- CBD and/or softening protein sequence of interest, or

2) Signal sequence -- (pro-peptide) – CBD and/or softening protein sequence of interest -- linker -- carbohydrate-binding domain,

in which the pro-peptide sequence normally contains 5-100, e.g. 5-25, amino acid residues. The recombinant product may be glycosylated or non-glycosylated.

Linking region

The term "linker" or "linking region" or "Middle region - MR" is intended to indicate a region that might adjoin the CBD and connect it to another CBD or to the amino acid sequence of a softening protein. When present, this linking can be achieved chemically or by recombinant techniques.

An example of the recombinant technique describing the expression of an enzyme with the CBD of different origin is described in S. Karita et al., (1996) Journal of Fermentation and Bioengineering, Vol. 81, No. 6, pp. 553-556. Preferred linking regions are amino acid linking regions (peptides), some examples thereof are described in N.R. Gilkes et al., Microbiol. Rev. 55, 1991, pp. 303-315. The linking region can comprise from 1 to about 100 amino acid residues, in particular of from 2 to 40 amino acid residues, e.g. from 2 to 15 amino acid residues. As stated above, it is preferred to use amino acids which are less favoured by the surrounding proteases.

Non amino acid/proteinic compounds, referred to as "non-amino acid" can also be used for the linking of the catalytically active amino acid sequence to the

CBD. Suitable non-amino acid linking regions are the polyethylene glycol derivatives described in the Shearwater polymers, Inc. catalog of January 1996, such as the nucleophilic PEGs, the carboxyl PEGs, the electrophilically activated PEGs, the sulfhydryl-selective PEGs, the heterofunctional PEGs, the biotin PEGs, the vinyl derivatives, the PEG silanes and the PEG phospholipids. In particular, suitable non-amino acid linking regions are the heterofunctional PEG, (X-PEG-Y) polymers from Shearwater such as PEG(NPC)2, PEG-(NH2)2, t-BOC-NH-PEG-Nh2, t-BOC-NH-PEG-CO2NHS, OH-PEG-NH-tBOC, FMOC-NH-PEG-CO2NHS or $PEG(NPC)_2$ MW 3400 from Sigma, glutaric dialdehyde 50 wt% solution in water from Aldrich, disuccinimidyl suberate (DSS) form Sigma, γ-maleimidobutyric acid N-hydroxysuccinimide ester (GMBS) from Sigma, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) from Sigma and dimethyl suberimidate hydrochloride (DMS) from Sigma.

Other suitable non-amino acid linking regions are 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide, N-ethyl-5-phenylisoaxolium-3-sulphonate, 1-cyclohexyl-3(2morpholinoethyl) carbodide metho-p-toluene sulphonate, N-ethoxycarbonyl-2-ethoxy 1,2, dihydroquinoline or glutaraldehyde.

Preferred chemical linking regions are PEG(NPC)2, (NH2)2-PEG, t-BOC-NH-PEG-NH2 polymers from Shearwater.

Fabric care and detergent components

Preferably, the fabric care compositions of the invention will contain at least one additional fabric care component. The precise nature of these additional components, and levels of incorporation thereof will depend on the physical form of the composition, and the nature of the cleaning operation for which it is to be used.

The composition may comprise optional ingredients such as a dye fixing agent, a fabric softener compound and further optional ingredients.The fabric care compositions of the present invention preferably further comprise a fabric care ingredient selected from cationic surfactants, a transferase enzyme and/or clays.

The composition of the invention can be employed in stand alone product including pre-or post-wash additives. It can also be employed It can also be used in fully-formulated compositions including laundry compositions as well as rinse

added fabric softener compositions and dryer added compositions (e.g. sheets) which provide softening and/or antistatic benefits, and rinse added compositions.

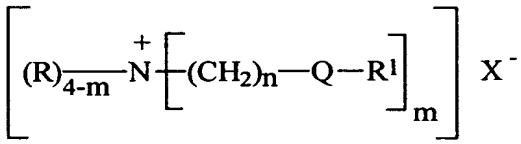
Cationic softeners

The fabric care compositions of the present invention preferably further comprise a cationic surfactant. It has been surprisingly found that the fabric care compositions of the present invention further comprising a cationic surfactant, can provide improved fabric softness and provide, refurbish or restore enhanced tensile strength, anti-wrinkle, anti-bobbling and anti-shrinkage properties to fabrics, as well as provide improved static control, colour appearance and fabric anti-wear properties and benefits.

Typical of the cationic softening components are the quaternary ammonium compounds or amine precursors thereof as defined hereinafter.

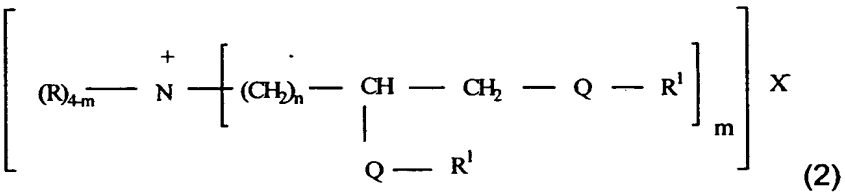
Quaternary Ammonium Fabric Softening Active Compound

(1) Preferred quaternary ammonium fabric softening active compound have the formula



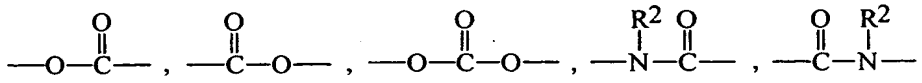
(1)

or the formula:



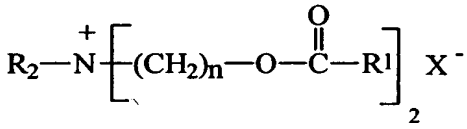
(2)

wherein Q is a functional unit having the formula:



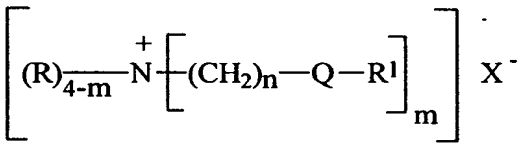
each R unit is independently hydrogen, C_1-C_6 alkyl, C_1-C_6 hydroxyalkyl, and mixtures thereof, preferably methyl or hydroxy alkyl; each R^1 unit is independently linear or branched C_{11}-C_{22} alkyl, linear or branched C_{11}-C_{22} alkenyl, and mixtures thereof, R^2 is hydrogen, C_1-C_4 alkyl, C_1-C_4 hydroxyalkyl, and mixtures thereof; X is an anion which is compatible with fabric softener actives and adjunct ingredients; the index m is from 1 to 4, preferably 2; the index n is from 1 to 4, preferably 2.

An example of a preferred fabric softener active is a mixture of quaternized amines having the formula:

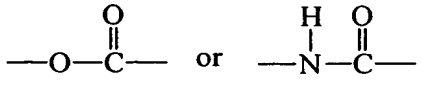


wherein R is preferably methyl; R^1 is a linear or branched alkyl or alkenyl chain comprising at least 11 atoms, preferably at least 15 atoms. In the above fabric softener example, the unit $-R^1$ represents a fatty alkyl or alkenyl unit which is typically derived from a triglyceride source. The triglyceride source is preferably derived from tallow, partially hydrogenated tallow, lard, partially hydrogenated lard, vegetable oils and/or partially hydrogenated vegetable oils, such as, canola oil, safflower oil, peanut oil, sunflower oil, corn oil, soybean oil, tall oil, rice bran oil, etc. and mixtures of these oils.

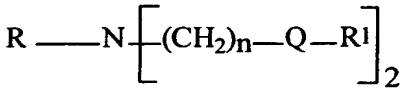
The preferred fabric softening actives of the present invention are the Diester and/or Diamide Quaternary Ammonium (DEQA) compounds, the diesters and diamides having the formula:



wherein R, R^1, X, and n are the same as defined herein above for formulas (1) and (2), and Q has the formula:

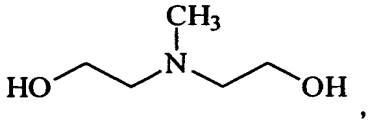


These preferred fabric softening actives are formed from the reaction of an amine with a fatty acyl unit to form an amine intermediate having the formula:

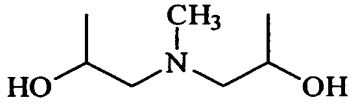


wherein R is preferably methyl, Q and R^1 are as defined herein before; followed by quaternization to the final softener active.

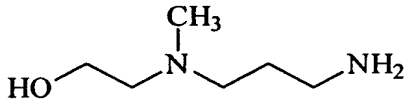
Non-limiting examples of preferred amines which are used to form the DEQA fabric softening actives according to the present invention include methyl bis(2-hydroxyethyl)amine having the formula:



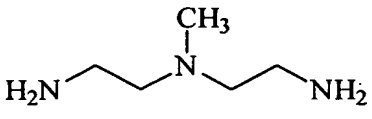
methyl bis(2-hydroxypropyl)amine having the formula:



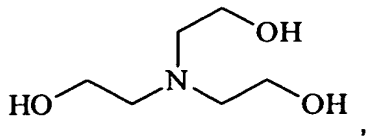
methyl (3-aminopropyl) (2-hydroxyethyl)amine having the formula:



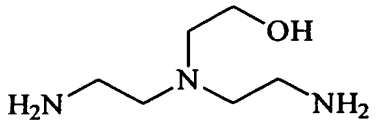
methyl bis(2-aminoethyl)amine having the formula:



triethanol amine having the formula:



di(2-aminoethyl) ethanolamine having the formula:



The counterion, $X^{(-)}$ above, can be any softener-compatible anion, preferably the anion of a strong acid, for example, chloride, bromide, methylsulfate, ethylsulfate, sulfate, nitrate and the like, more preferably chloride or methyl sulfate. The anion can also, but less preferably, carry a double charge in which case $X^{(-)}$ represents half a group.

Tallow and canola oil are convenient and inexpensive sources of fatty acyl units which are suitable for use in the present invention as R^1 units. The following are non-limiting examples of quaternary ammonium compounds suitable for use in the compositions of the present invention. The term "tallowyl" as used herein below indicates the R^1 unit is derived from a tallow triglyceride source and is a mixture of fatty alkyl or alkenyl units. Likewise, the use of the term canolyl refers to a mixture of fatty alkyl or alkenyl units derived from canola oil.

In the following table are described non-limiting examples of suitable fabric softener according to the above formula. In this list, the term "oxy" defines a —C(=O)— unit, whereas the term "oxo" defines a -O- unit.

Table II

Fabric Softener Actives

N,N-di(tallowyl-oxy-2-oxo-ethyl)-N-methyl, N-(2-hydroxyethyl) ammonium chloride;

N,N-di(canolyl-oxy-2-oxo-ethyl)-N-methyl, N-(2-hydroxyethyl) ammonium chloride;
N,N-di(tallowyloxy-2-oxo-ethyl)-N,N-dimethyl ammonium chloride;
N,N-di(canolyloxy-2-oxo-ethyl)-N,N-dimethyl ammonium chloride
N,N,N-tri(tallowyl-oxy-2-oxo-ethyl)-N-methyl ammonium chloride;
N,N,N-tri(canolyl-oxy-2-oxo-ethyl)-N-methyl ammonium chloride;
N-(tallowyloxy-2-oxo-ethyl)-N-(tallowyl)-N,N-dimethyl ammonium chloride;
N-(canolyloxy-2-oxo-ethyl)-N-(canolyl)-N,N-dimethyl ammonium chloride;
1,2-di(tallowyloxy-oxo)-3-N,N,N-trimethylammoniopropane chloride; and
1,2-di(canolyloxy-oxo)-3-N,N,N-trimethylammoniopropane chloride;
and mixtures of the above actives.

Other examples of quaternay ammoniun softening compounds are methylbis(tallowamidoethyl)(2-hydroxyethyl)ammonium methylsulfate and methylbis(hydrogenated tallowamidoethyl)(2-hydroxyethyl)ammonium methyl-sulfate; these materials are available from Witco Chemical Company under the trade names Varisoft® 222 and Varisoft® 110, respectively.

Particularly preferred is N,N-di(tallowyl-oxy-2-oxo-ethyl)-N-methyl, N-(2-hydroxyethyl) ammonium chloride, where the tallow chains are at least partially unsaturated.

Transferase enzyme

The fabric care compositions of the present invention preferably further comprise a transferase enzyme. It has been surprisingly found that the fabric care compositions of the present invention further comprising a transferase enzyme, can provide improved fabric softness and provide, refurbish or restore enhanced tensile strength, anti-wrinkle, anti-bobbling and anti-shrinkage properties to fabrics, as well as provide improved static control, colour appearance and fabric anti-wear properties and benefits

Transferase enzymes catalyse the transfer of functional compounds to a range of substrates. Particularly, the transferase of the invention have the potential to transfer a chemical moiety, for example a methyl group or a glycosyl

group, from a small substrate to form oligomeric molecules or elongate polymeric compounds. Using small substrates, the enzyme improves the properties of garments by binding functional groups like methyl, hydroxymethyl, formyl, carboxyl, aldehyde, ketone, acyl, amino and phosphorous functional groups and/or transferring glycosyl residues to the garment surface.

Without wishing to be bound by theory, it is believed that aminoacyl transferase of the IUPAC Classification EC 2.3.2 links the amino acid(s) of the CBDs, Cross-linked CBDs, the amino acid linking regions and/or the softening proteins to the cotton fibres of the fabric and thereby provide, refurbish or restore tensile strength. Moreover, it is believed that glycosyl transferase of the IUPAC Classification EC 2.4 transfer and link covalently the glycosyl carbohydrates that can be found on the CBDs, Cross-linked CBDs, the amino acid linking regions and/or the softening proteins to the fabric and thereby provide, refurbish or restore tensile strength.

The aminoacyl transferases (EC 2.3.2) are enzymes transferring amino groups from a donor, generally an amino acid, to an acceptor. Even more preferred is the protein-glutamine γ-glutamyltransferase (EC 2.3.2.13), also available under the name transglutaminase.

The general properties of the glycosyltransferases is to transfer a sugar from oligosaccharides to another carbohydrate as acceptor. Both hexosyltransferases and pentosyltransferases can be used in the invention.

Examples of suitable glycosyltransferases are galactosyl transferases and fructosyltransferases, such as 1,4-β-galactosyltransferase; 1,3-α-fructosyl-transferase; 2,3-sialyl transferase; cyclodextrin glycosyltransferase; N-acetylgluco- or -galactosaminyltransferase. Of particular interest is EC 2.4.1.24 1,4-α-D-glucan : 1,4-a-D-glucan(D-glucose) 6-α-D-glucosyl transferase. A particulate member of this enzyme is commercially available under the name Transglucosidase L-500.

In addition to the glycosyltransferases discussed above, it has been found that mutant glycosyltransferases and/or glycosidase, examples of which are described in PCT Application Publication No. WO 97/21822 to S.G. Withers Protein Eng. Net. Canada, improve the tensile strength and appearance of

fabrics, e.g., reduce fabric wrinkles, enhance shape retention and reduce shrinkage

Yet another enzyme that is of particular interest is cyclomaltodextrin glucanotransferase ("CGT-ase") (EC 2.4.1.19), which is commercially available from Amano and Novo Nordisk A/S.

Yet still another group of enzymes that is of particular interest is glucansucrases, of which dextransucrase (EC 2.4.1.5), a glycosyltransferase, is one example. Other glucansucrases that are suitable for use in the compositions described herein include, but are not limited to, various dextransucrases, alternansucrase and levansucrase. Levansucrase is commercially available from Genencor.

These transferases are preferably incorporated into the fabric care compositions in accordance with the invention at a level of from 0.0001% to 10%, more preferably from 0.0005% to 5 %, most preferred from 0.001% to 1% pure enzyme by weight of the total composition.

The above-mentioned enzymes may be of any suitable origin, such as vegetable, animal, bacterial, fungal and yeast origin. Origin can further be mesophilic or extremophilic (psychrophilic, psychrotrophic, thermophilic, barophilic, alkalophilic, acidophilic, halophilic, etc.). Purified or non-purified forms of these enzymes may be used. Nowadays, it is common practice to modify wild-type enzymes via protein / genetic engineering techniques in order to optimise their performance efficiency in the fabric care compositions of the invention. For example, the variants may be designed such that the compatibility of the enzyme to commonly encountered ingredients of such compositions is increased. Alternatively, the variant may be designed such that the optimal pH, bleach and/or chelant stability, catalytic activity and the like, of the enzyme variant is tailored to suit the particular fabric conditioning and/or cleaning application.

In particular, attention should be focused on amino acids sensitive to oxidation in the case of bleach stability and on surface charges for the surfactant compatibility. The isoelectric point of such enzymes may be modified by the substitution of some charged amino acids, e.g. an increase in isoelectric point may help to improve compatibility with anionic surfactants. The stability of the enzymes may be further enhanced by the creation of e.g. additional salt bridges and enforcing calcium binding sites to increase chelant stability.

Colour care and fabric care benefits

Technologies which provide a type of colour care benefit can also be included. Examples of these technologies are metallo catalysts for colour maintenance. Such metallo catalysts are described in copending European Patent Application No. 92870181.2. Dye fixing agents, polyolefin dispersion for anti-wrinkles and improved water absorbancy, perfume and amino-functional polymer (PCT/US97/16546) for colour care treatment and perfume substantivity are further examples of colour care / fabric care technologies and are described in the co-pending Patent Application No. 96870140.9, filed November 07, 1996.

Fabric softening agents can also be incorporated into fabric care compositions in accordance with the present invention. These agents may be inorganic or organic in type. Inorganic softening agents are exemplified by the smectite clays disclosed in GB-A-1 400 898 and in USP 5,019,292. Organic fabric softening agents include the water insoluble tertiary amines as disclosed in GB-A1 514 276 and EP-B0 011 340 and their combination with mono C12-C14 quaternary ammonium salts are disclosed in EP-B-0 026 527 and EP-B-0 026 528 and di-long-chain amides as disclosed in EP-B-0 242 919. Other useful organic ingredients of fabric softening systems include high molecular weight polyethylene oxide materials as disclosed in EP-A-0 299 575 and 0 313 146.

Preferably, the fabric care compositions of the present invention will comprise a clay. It has been surprisingly found that the fabric care compositions of the present invention further comprising a clay, can provide improved fabric softness and provide, refurbish or restore enhanced tensile strength, anti-wrinkle, anti-bobbling and anti-shrinkage properties to fabrics, as well as provide improved static control, colour appearance and fabric anti-wear properties and benefits.

Levels of smectite clay are normally in the range from 2% to 20%, more preferably from 5% to 15% by weight. Organic fabric softening agents such as the water-insoluble tertiary amines or dilong chain amide materials are incorporated at levels of from 0.5% to 5% by weight, normally from 1% to 3% by weight whilst the high molecular weight polyethylene oxide materials and the

water soluble cationic materials are added at levels of from 0.1% to 2%, normally from 0.15% to 1.5% by weight.

Dye fixing agent

The composition of the invention may optionally comprise a dye fixing agent. Dye fixing agents, or "fixatives", are well-known, commercially available materials which are designed to improve the appearance of dyed fabrics by minimizing the loss of dye from fabrics due to washing. Not included within this definition are components which are fabric softeners or those described hereinbefore as amino-functional polymers.

Many dye fixing agents are cationic, and are based on various quaternized or otherwise cationically charged organic nitrogen compounds. Cationic fixatives are available under various trade names from several suppliers. Representative examples include: CROSCOLOR PMF (July 1981, Code No. 7894) and CROSCOLOR NOFF (January 1988, Code No. 8544) from Crosfield; INDOSOL E-50 (February 27, 1984, Ref. No. 6008.35.84; polyethyleneamine-based) from Sandoz; SANDOFIX TPS, which is also available from Sandoz and is a preferred polycationic fixative for use herein and SANDOFIX SWE (cationic resinous compound), REWIN SRF, REWIN SRF-O and REWIN DWR from CHT-Beitlich GMBH, Tinofix® ECO, Tinofix®FRD and Solfin® available from Ciba-Geigy.

Other cationic dye fixing agents are described in "Aftertreatments for improving the fastness of dyes on textile fibres" by Christopher C. Cook (REV. PROG. COLORATION Vol. 12, 1982). Dye fixing agents suitable for use in the present invention are ammonium compounds such as fatty acid - diamine condensates e.g. the hydrochloride, acetate, metosulphate and benzyl hydrochloride of oleyldiethyl aminoethylamide, oleylmethyl-diethylenediaminemethosulphate, monostearyl-ethylene diaminotrimethyl-ammonium methosulphate and oxidized products of tertiary amines; derivatives of polymeric alkyldiamines, polyamine-cyanuric chloride condensates and aminated glycerol dichlorohydrins.

A typical amount of the dye fixing agent to be employed in the composition of the invention is preferably up 90% by weight, preferably up to 50% by weight,

more preferably from 0.001% to 10% by weight, most preferably from 0.5% to 5% active by weight of the composition.

Fabric softening compound

Typical levels of incorporation of the softening compound in the fabric care composition are of from 1% to 80% by weight, preferably from 5% to 75%, more preferably from 15% to 70%, and even more preferably from 19% to 65%, by weight of the composition.

The fabric softener compound is preferably selected from a cationic, nonionic, amphoteric or anionic fabric softening component. Typical of the cationic softening components are the quaternary ammonium compounds or amine precursors thereof as defined hereinafter.

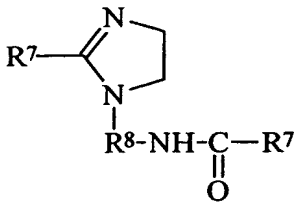
Quaternary Ammonium Fabric Softening Active Compound

As described above, the fabric care compositions of the present invention preferably further comprise a cationic surfacants.

Amine Fabric Softening Active Compound

Suitable amine fabric softening compounds for use herein, which may be in amine form or cationic form are selected from:

(i)- Reaction products of higher fatty acids with a polyamine selected from the group consisting of hydroxyalkylalkylenediamines and dialkylenetriamines and mixtures thereof. These reaction products are mixtures of several compounds in view of the multi-functional structure of the polyamines.
The preferred Component (i) is a nitrogenous compound selected from the group consisting of the reaction product mixtures or some selected components of the mixtures.
One preferred component (i) is a compound selected from the group consisting of substituted imidazoline compounds having the formula:



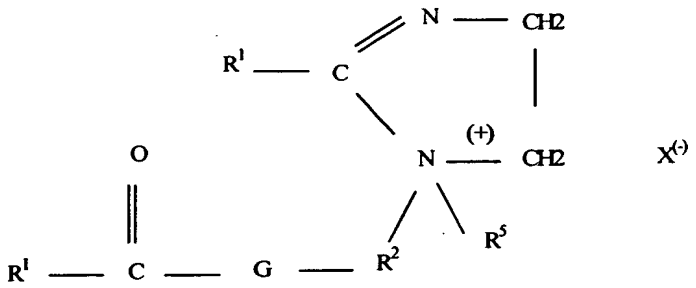
wherein R^7 is an acyclic aliphatic C_{15}-C_{21} hydrocarbon group and R^8 is a divalent C_1-C_3 alkylene group.

Component (i) materials are commercially available as: Mazamide® 6, sold by Mazer Chemicals, or Ceranine® HC, sold by Sandoz Colors & Chemicals; stearic hydroxyethyl imidazoline sold under the trade names of Alkazine® ST by Alkaril Chemicals, Inc., or Schercozoline® S by Scher Chemicals, Inc.; N,N"-ditallowalkoyldiethylenetriamine; 1-tallowamidoethyl-2-tallowimidazoline (wherein in the preceding structure R^1 is an aliphatic C_{15}-C_{17} hydrocarbon group and R^8 is a divalent ethylene group).

Certain of the Components (i) can also be first dispersed in a Bronsted acid dispersing aid having a pKa value of not greater than about 4; provided that the pH of the final composition is not greater than about 6. Some preferred dispersing aids are hydrochloric acid, phosphoric acid, or methylsulfonic acid.

Both N,N"-ditallowalkoyldiethylenetriamine and 1-tallow(amidoethyl)-2-tallowimidazoline are reaction products of tallow fatty acids and diethylenetriamine, and are precursors of the cationic fabric softening agent methyl-1-tallowamidoethyl-2-tallowimidazolinium methylsulfate (see "Cationic Surface Active Agents as Fabric Softeners," R. R. Egan, Journal of the American Oil Chemicals' Society, January 1978, pages 118-121). N,N"-ditallow alkoyldiethylenetriamine and 1-tallowamidoethyl-2-tallowimidazoline can be obtained from Witco Chemical Company as experimental chemicals. Methyl-1-tallowamidoethyl-2-tallowimidazolinium methylsulfate is sold by Witco Chemical Company under the tradename Varisoft® 475.

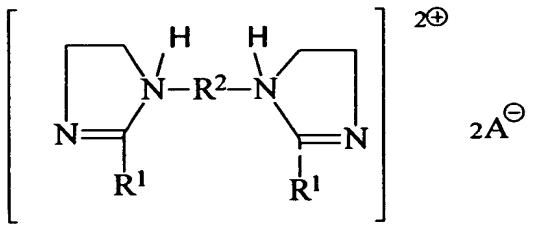
(ii)-softener having the formula:



wherein each R^2 is a C_{1-6} alkylene group, preferably an ethylene group; and G is an oxygen atom or an -NR- group; and each R, R^1, R^2 and R^5 have the definitions given above and A^- has the definitions given above for X^-.

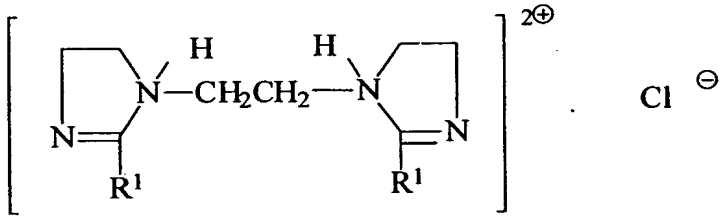
An example of Compound (ii) is 1-oleylamidoethyl-2-oleylimidazolinium chloride wherein R^1 is an acyclic aliphatic C_{15}-C_{17} hydrocarbon group, R^2 is an ethylene group, G is a NH group, R^5 is a methyl group and A^- is a chloride anion.

(iii)- softener having the formula:



wherein R, R^1, R^2, and A^- are defined as above.

An example of Compound (iii) is the compound having the formula:



wherein R^1 is derived from oleic acid.

Additional fabric softening agents useful herein are described in U.S. Pat. No. 4,661,269, issued April 28, 1987, in the names of Toan Trinh, Errol H. Wahl, Donald M. Swartley, and Ronald L. Hemingway; U.S. Pat. No. 4,439,335, Burns,

issued March 27, 1984; and in U.S. Pat. Nos.: 3,861,870, Edwards and Diehl; 4,308,151, Cambre; 3,886,075, Bernardino; 4,233,164, Davis; 4,401,578, Verbruggen; 3,974,076, Wiersema and Rieke; 4,237,016, Rudkin, Clint, and Young; and European Patent Application publication No. 472,178, by Yamamura et al., all of said documents being incorporated herein by reference.

Of course, the term "softening active" can also encompass mixed softening active agents. Preferred among the classes of softener compounds disclosed herein before are the diester or diamido quaternary ammonium fabric softening active compound (DEQA).

Fully formulated fabric care compositions may contain, in addition to the hereinbefore described components, one or more of the following ingredients.

Liquid carrier

Another optional, but preferred, ingredient is a liquid carrier. The liquid carrier employed in the instant compositions is preferably at least primarily water due to its low cost, relative availability, safety, and environmental compatibility. The level of water in the liquid carrier is preferably at least about 50%, most preferably at least about 60%, by weight of the carrier. Mixtures of water and low molecular weight, e.g., <about 200, organic solvent, e.g., lower alcohols such as ethanol, propanol, isopropanol or butanol are useful as the carrier liquid. Low molecular weight alcohols include monohydric, dihydric (glycol, etc.) trihydric (glycerol, etc.), and higher polyhydric (polyols) alcohols.

Additional Solvents

The compositions of the present invention may comprise one or more solvents which provide increased ease of formulation. These ease of formulation solvents are all disclosed in WO 97/03169. This is particularly the case when formulating liquid, clear fabric softening compositions. When employed, the ease of formulation solvent system preferably comprises less than about 40%, preferably from about 10% to about 35%, more preferably from about 12% to about 25%, and even more preferably from about 14% to about 20%, by weight of the composition. The ease of formulation solvent is selected to minimize

solvent odor impact in the composition and to provide a low viscosity to the final composition. For example, isopropyl alcohol is not very effective and has a strong odor. n-Propyl alcohol is more effective, but also has a distinct odor. Several butyl alcohols also have odors but can be used for effective clarity/stability, especially when used as part of a ease of formulation solvent system to minimize their odor. The alcohols are also selected for optimum low temperature stability, that is they are able to form compositions that are liquid with acceptable low viscosities and translucent, preferably clear, down to about 40°F (about 4.4°C) and are able to recover after storage down to about 20°F (about minus 6.7°C).

The suitability of any ease of formulation solvent for the formulation of the liquid, concentrated, preferably clear, fabric softener compositions herein with the requisite stability is surprisingly selective. Suitable solvents can be selected based upon their octanol/water partition coefficient (P) as defined in WO 97/03169.

The ease of formulation solvents herein are selected from those having a ClogP of from about 0.15 to about 0.64, preferably from about 0.25 to about 0.62, and more preferably from about 0.40 to about 0.60, said ease of formulation solvent preferably being at least somewhat asymmetric, and preferably having a melting, or solidification, point that allows it to be liquid at, or near room temperature. Solvents that have a low molecular weight and are biodegradable are also desirable for some purposes. The more assymetric solvents appear to be very desirable, whereas the highly symmetrical solvents such as 1,7-heptanediol, or 1,4-bis(hydroxymethyl) cyclohexane, which have a center of symmetry, appear to be unable to provide the essential clear compositions when used alone, even though their ClogP values fall in the preferred range.

The most preferred ease of formulation solvents can be identified by the appearance of the softener vesicles, as observed via cryogenic electron microscopy of the compositions that have been diluted to the concentration used in the rinse. These dilute compositions appear to have dispersions of fabric softener that exhibit a more unilamellar appearance than conventional fabric softener compositions. The closer to uni-lamellar the appearance, the better the compositions seem to perform. These compositions provide surprisingly good

fabric softening as compared to similar compositions prepared in the conventional way with the same fabric softener active.

Operable ease of formulation solvents are disclosed and listed below which have ClogP values which fall within the requisite range. These include mono-ols, C6 diols, C7 diols, octanediol isomers, butanediol derivatives, trimethylpentanediol isomers, ethylmethylpentanediol isomers, propyl pentanediol isomers, dimethylhexanediol isomers, ethylhexanediol isomers, methylheptanediol isomers, octanediol isomers, nonanediol isomers, alkyl glyceryl ethers, di(hydroxy alkyl) ethers, and aryl glyceryl ethers, aromatic glyceryl ethers, alicyclic diols and derivatives, C_3C_7 diol alkoxylated derivatives, aromatic diols, and unsaturated diols. Particularly preferred ease of formulation solvents include hexanediols such as 1,2-Hexanediol and 2-Ethyl-1,3-hexanediol and pentanediols such as 2,2,4-Trimethyl-1,3-pentanediol.

Dispersibility Aids

Relatively concentrated compositions containing both saturated and unsaturated diester quaternary ammonium compounds can be prepared that are stable without the addition of concentration aids. However, the compositions of the present invention may require organic and/or inorganic concentration aids to go to even higher concentrations and/or to meet higher stability standards depending on the other ingredients. These concentration aids which typically can be viscosity modifiers may be needed, or preferred, for ensuring stability under extreme conditions when particular softener active levels are used. The surfactant concentration aids are typically selected from the group consisting of (1) single long chain alkyl cationic surfactants; (2) nonionic surfactants; (3) amine oxides; (4) fatty acids; and (5) mixtures thereof. These aids are described in WO 94/20597, specifically on page 14, line 12 to page 20, line 12, which is herein incorporated by reference.

When said dispersibility aids are present , the total level is from 2% to 25%, preferably from 3% to 17%, more preferably from 4% to 15%, and even more preferably from 5% to 13% by weight of the composition. These materials can either be added as part of the active softener raw material, (I), e.g., the mono-long chain alkyl cationic surfactant and/or the fatty acid which are reactants used to form the biodegradable fabric softener active as discussed hereinbefore, or

added as a separate component. The total level of dispersibility aid includes any amount that may be present as part of component (I).

Inorganic viscosity/dispersibility control agents which can also act like or augment the effect of the surfactant concentration aids, include water-soluble, ionizable salts which can also optionally be incorporated into the compositions of the present invention. A wide variety of ionizable salts can be used. Examples of suitable salts are the halides of the Group IA and IIA metals of the Periodic Table of the Elements, e.g., calcium chloride, magnesium chloride, sodium chloride, potassium bromide, and lithium chloride. The ionizable salts are particularly useful during the process of mixing the ingredients to make the compositions herein, and later to obtain the desired viscosity. The amount of ionizable salts used depends on the amount of active ingredients used in the compositions and can be adjusted according to the desires of the formulator. Typical levels of salts used to control the composition viscosity are from about 20 to about 20,000 parts per million (ppm), preferably from about 20 to about 11,000 ppm, by weight of the composition.

Alkylene polyammonium salts can be incorporated into the composition to give viscosity control in addition to or in place of the water-soluble, ionizable salts above. In addition, these agents can act as scavengers, forming ion pairs with anionic detergent carried over from the main wash, in the rinse, and on the fabrics, and may improve softness performance. These agents may stabilize the viscosity over a broader range of temperature, especially at low temperatures, compared to the inorganic electrolytes.

Specific examples of alkylene polyammonium salts include l-lysine monohydrochloride and 1,5-diammonium 2-methyl pentane dihydrochloride.

Stabilizers

Stabilizers can be present in the compositions of the present invention. The term "stabilizer," as used herein, includes antioxidants and reductive agents. These agents are present at a level of from 0% to about 2%, preferably from about 0.01% to about 0.2%, more preferably from about 0.035% to about 0.1% for antioxidants, and more preferably from about 0.01% to about 0.2% for reductive agents. These assure good odor stability under long term storage conditions for the compositions and compounds stored in molten form. The use

of antioxidants and reductive agent stabilizers is especially critical for low scent products (low perfume).

Examples of antioxidants that can be added to the compositions of this invention include a mixture of ascorbic acid, ascorbic palmitate, propyl gallate, available from Eastman Chemical Products, Inc., under the trade names Tenox® PG and Tenox S-1; a mixture of BHT (butylated hydroxytoluene), BHA (butylated hydroxyanisole), propyl gallate, and citric acid, available from Eastman Chemical Products, Inc., under the trade name Tenox-6; butylated hydroxytoluene, available from UOP Process Division under the trade name Sustane® BHT; tertiary butylhydroquinone, Eastman Chemical Products, Inc., as Tenox TBHQ; natural tocopherols, Eastman Chemical Products, Inc., as Tenox GT-1/GT-2; and butylated hydroxyanisole, Eastman Chemical Products, Inc., as BHA; long chain esters (C_8-C_{22}) of gallic acid, e.g., dodecyl gallate; Irganox® 1010; Irganox® 1035; Irganox® B 1171; Irganox® 1425; Irganox® 3114; Irganox® 3125; and mixtures thereof; preferably Irganox® 3125, Irganox® 1425, Irganox® 3114, and mixtures thereof; more preferably Irganox® 3125 alone. The chemical names and CAS numbers for some of the above stabilizers are listed in Table II below.

TABLE II

Antioxidant	CAS No.	Chemical Name used in Code of Federal Regulations
Irganox® 1010	6683-19-8	Tetrakis (methylene(3,5-di-tert-butyl-4 hydroxyhydrocinnamate)) methane
Irganox® 1035	41484-35-9	Thiodiethylene bis(3,5-di-tert-butyl-4-hydroxyhydrocinnamate
Irganox® 1098	23128-74-7	N,N'-Hexamethylene bis(3,5-di-tert-butyl-4-hydroxyhydrocinnamamide
Irganox® B 1171	31570-04-4 23128-74-7	1:1 Blend of Irganox® 1098 and Irgafos® 168
Irganox® 1425	65140-91-2	Calcium bis(monoethyl(3,5-di-tert-butyl-4-hydroxybenzyl)phosphonate)
Irganox® 3114	65140-91-2	Calcium bis(monoethyl(3,5-di-tert-butyl-4-hydroxybenzyl)phosphonate)
Irganox® 3125	34137-09-2	3,5-Di-tert-butyl-4-hydroxy-hydrocinnamic acid

		triester with 1,3,5-tris(2-hydroxyethyl)-S-triazine-2,4,6-(1H, 3H, 5H)-trione
Irgafos® 168	31570-04-4	Tris(2,4-di-tert-butyl-phenyl)phosphite

Examples of reductive agents include sodium borohydride, hypophosphorous acid, Irgafos® 168, and mixtures thereof.

Soil Release Agent

Soil Release agents are desirably used in fabric care compositions of the instant invention. Any polymeric soil release agent known to those skilled in the art can optionally be employed in the compositions of this invention. Polymeric soil release agents are characterized by having both hydrophilic segments, to hydrophilize the surface of hydrophobic fibers, such as polyester and nylon, and hydrophobic segments, to deposit upon hydrophobic fibers and remain adhered thereto through completion of washing and rinsing cycles and, thus, serve as an anchor for the hydrophilic segments. This can enable stains occurring subsequent to treatment with the soil release agent to be more easily cleaned in later washing procedures.

If utilized, soil release agents will generally comprise from about 0.01% to about 10.0%, by weight, of the fabric care compositions herein, typically from about 0.1% to about 5%, preferably from about 0.2% to about 3.0%.

The following, all included herein by reference, describe soil release polymers suitable for use in the present invention. U.S. 3,959,230 Hays, issued May 25, 1976; U.S. 3,893,929 Basadur, issued July 8, 1975; U.S. 4,000,093, Nicol, et al., issued December 28, 1976; U.S. Patent 4,702,857 Gosselink, issued October 27, 1987; U.S. 4,968,451, Scheibel et al., issued November 6; U.S. 4,702,857, Gosselink, issued October 27, 1987; U.S. 4,711,730, Gosselink et al., issued December 8, 1987; U.S. 4,721,580, Gosselink, issued January 26, 1988; U.S. 4,877,896, Maldonado et al., issued October 31, 1989; U.S. 4,956,447, Gosselink et al., issued September 11, 1990; U.S. 5,415,807 Gosselink et al., issued May 16, 1995; European Patent Application 0 219 048, published April 22, 1987 by Kud, et al..

Further suitable soil release agents are described in U.S. 4,201,824, Violland et al.; U.S. 4,240,918 Lagasse et al.; U.S. 4,525,524 Tung et al.; U.S. 4,579,681, Ruppert et al.; U.S. 4,240,918; U.S. 4,787,989; U.S. 4,525,524; EP 279,134 A, 1988, to Rhone-Poulenc Chemie; EP 457,205 A to BASF (1991); and DE 2,335,044 to Unilever N. V., 1974 all incorporated herein by reference.

Commercially available soil release agents include the METOLOSE SM100, METOLOSE SM200 manufactured by Shin-etsu Kagaku Kogyo K.K., SOKALAN type of material, e.g., SOKALAN HP-22, available from BASF (Germany), ZELCON 5126 (from Dupont) and MILEASE T (from ICI).

Bactericides

Examples of bactericides used in the compositions of this invention include glutaraldehyde, formaldehyde, 2-bromo-2-nitro-propane-1,3-diol sold by Inolex Chemicals, located in Philadelphia, Pennsylvania, under the trade name Bronopol®, and a mixture of 5-chloro-2-methyl-4-isothiazoline-3-one and 2-methyl-4-isothiazoline-3-one sold by Rohm and Haas Company under the trade name Kathon 1 to 1,000 ppm by weight of the agent.

Perfume

The present invention can contain a perfume. Suitable perfumes are disclosed in U.S. Pat. 5,500,138, said patent being incorporated herein by reference.

As used herein, perfume includes fragrant substance or mixture of substances including natural (i.e., obtained by extraction of flowers, herbs, leaves, roots, barks, wood, blossoms or plants), artificial (i.e., a mixture of different nature oils or oil constituents) and synthetic (i.e., synthetically produced) odoriferous substances. Such materials are often accompanied by auxiliary materials, such as fixatives, extenders, stabilizers and solvents. These auxiliaries are also included within the meaning of "perfume", as used herein. Typically, perfumes are complex mixtures of a plurality of organic compounds.